Plant Pathology Circular No. 351
January-February 1992

Fla. Dept. Agric. & Consumer Services
Division of Plant Industry

STUBBORN DISEASE OF CITRUS Lawrence G. Brown[1]

Spiroplasma cirri Saglio et al, the causal agent of stubborn disease of citrus, is not known to occur in Florida. It was first reported in 1915 in California, but it was not until 1942 that stubborn was considered an infectious virus-like disease (6) and even later before the true nature of this mycoplasmalike pathogen was demonstrated. *S. citri is* of regulatory concern to the State of Florida, and programs are in place to prevent entry of this and other harmful citrus pathogens. The Citrus Germplasm Introduction Program provides the Florida citrus industry germplasm sources for commercial application and/or research purposes. The germplasm is indexed for all known virus and virus-like pathogens before it is released.

SYMPTOMS: Symptoms of stubborn are expressed most frequently under warm conditions, and even highly susceptible hosts may remain symptomless in cool weather. Stubborn is rarely lethal in citrus, but trees affected while young are often severely stunted. The internodes are shortened, and the foliage is dense and abnormally upright. Leaves may be cupped and unnaturally thick, and they frequently have variable chlorotic patterns which resemble nutritional deficiency. The mottled appearance and veinal chlorosis could be confused with symptoms caused by mechanical girdling, citrus tristeza virus, and/or the cachexia viroid. Stubborn-affected trees usually produce fewer and smaller fruit which are habitually lopsided or acorn-shaped. Fruits often do not color at the stem end as they mature, and seeds are frequently aborted. Symptoms on mature trees *are* usually less conspicuous (6).

HOST RANGE: Most citrus species and cultivars and a wide range of non-citrus plants are hosts of S. *citri (3,6).* Sweet oranges, grapefruit, tangelos, and mandarins and mandarin hybrids are highly susceptible to infection (6). Acid limes, lemons, trifoliate orange and trifoliate orange hybrids can be infected experimentally by graft inoculation, but usually exhibit milder symptoms than other varieties (6). Non-citrus hosts in which the pathogen has been found to occur naturally include periwinkle *(Catharanthus roseus L.),* London rocket *(Sisymbrium irio L.),* horseradish *(Armoracia rusticana* P. Gaertn, B. Mey, and Scherb.), many *Brassica* spp., and wild radish *(Raphanus raphanistrum* L.) (5,6).

DISTRIBUTION: *S. citri is* found in the western USA, Eastern Mediterranean, Middle East, North Africa, (3,6) and possibly Peru (7). It is an important disease in the warm and regions of California, Arizona, most of North Africa, the eastern Mediterranean Basin and the Middle East (6). The disease does not appear to be a problem in cool areas or areas with warm, humid climates (6). Even though the climate of Florida may not be conducive to disease development, a quarantine should still be maintained because there are no studies to confirm this observation.

Figure 1. Young citrus trees (arrows) showing stunting and dense foliage. Non-symptomatic tree in background.

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DISEASE DEVELOPMENT: *S. citri* is a motile, helically filamentous, mycoplasmalike organism that lacks a true cell wall (6). It is graft-transmissible but is neither seed nor mechanically transmissible (6). The elucidation of the mycoplasmalike nature of stubborn was important in the identification of the leafhopper, *Scaphytopius nitridus* (Deleing), as one of the insect vectors in California (1). *S. citri* is spread by S. *nitridus* and the beet leafhopper, *Circulifer tenellus* (Baker), in the southwest US (6). The only vector known to occur in Florida is *C. tenellus,* but it has only been collected on sea purslane, *Sesuvium portucastrum (L.)* L., in the brackish areas along the coast near Miami and in the Florida Keys which are outside the agricultural areas in Florida (2, F. Mead, DPI, personal communication). *Neoaliturus haemoceps* (Mulsant & Rey) is a suspected vector in the Mediterranean area (6). In regions where S. *citri* is established, the primary infections in citrus occur when the leafhopper vectors carrying the stubborn organism move onto citrus from plants other than citrus. The spread of the disease fluctuates with the size and make-up of the vector population, the type inoculum reservoir, and the season (6). It is most rapid and noticeable in young groves. Movement of the vector from other hosts onto citrus is seasonal and increases as natural vegetation dries up at the beginning of the dry summer seasons. The natural spread of the disease into citrus is most rapid and noticeable in young orchards. Secondary tree-to-tree spread is probably an economic factor only in young orchards that contain large numbers of infected trees. This may occur when infected budwood is used for propagation. Infection of only a few trees in a mature orchard may present little hazard to nearby trees (6).

MANAGEMENT: The greatest risk for spread of stubborn into Florida is by the use of non-indexed budwood. Stubborn has been excluded from Florida by the controlled importation of citrus germplasm, the use of disease-free budwood, and by inspection. In California the disease is managed by surveying, culling, and replanting. A favorable response to tetracycline treatment has been shown experimentally, but chemotherapy has not been used commercially (6).

SURVEY AND DETECTION: Symptoms of stubborn disease are variable and readily confused with other problems. Laboratory and greenhouse tests are required for positive identification (3,6,7). Field symptoms include a lack of fruiting, or small, lopsided fruit with aborted seeds. Other symptoms are severe stunting of young trees, shortened internodes and dense foliage with cupped, abnormally thick leaves showing variable chlorotic patterns (6). Surveys during warm weather are best because symptoms are most evident during periods when the daytime temperatures continually exceed 27 C (80 F) (6).

Laboratory confirmation can be done by grafting to citrus indicators and by culturing S. *citri*. The classic grafting approach utilizes young leaf pieces or side grafts onto Duncan or Marsh grapefruit, or Madam Vinous sweet orange seedlings in a warm (day 27-35 C, night 21-24 C) greenhouse (3,6,7). Three growth flushes should be observed for misshapen and mottled leaves, and seedling stunting. However, these symptoms are not always diagnostic. *S. citri* cultured on artificial media and identified by microscopy and/or serological and DNA probes is a better diagnostic method (6). However, as with grafting, the selection of tissue is important because of the uneven distribution of S. *citri* in citrus phloem. In liquid culture, the spiral filaments are frequently attached to irregularly shaped basal bodies, 1-2 µm in diameter (4,6). These aggregates can be recognized in liquid cultures examined by phase contrast or dark field microscopy. Characteristic "fried egg" colonies are formed on semisolid media (4,5).

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